Paratopic interaction

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Paratopic interaction, a mechanism in the generation of structure bound enzymatic activity

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Summary. A general mechanism is recognized that can cause specific enzymatic activity at interphases. It consists of 2 proteins bound in close juxtaposition at a micelle or membrane surface. One, the enzyme *sensu strictu*, bears the active site, the other, the paraenzyme, is essential for generation or specific modification of the enzymatic activity.

It is the purpose of this report to draw attention to a kind of interaction between protein molecules and an interface, that can regulate, or even generate, enzymatic activity. The basic unit of this concept consists of 2 different protein molecules adsorbed next to each other onto an interface. This configuration constitutes an enzymatically active moiety. The active site is present on one of the 2 molecules, called the active site carrier; the enzymatic activity, however, is governed by the presence of the second protein molecule, called the paraenzyme. For this kind of interaction we suggest the name

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Enzymatic activities generated by paratopic interaction

Surface	Active site carrier	Para-enzyme	Substrate	Product
Phospholipid micelle	Factor X_a	Factor V_a	Prothrombin	Thrombin
Phospholipid micelle	Factor IX_a	Factor $VIII_a$	Factor X	Factor X_a
Erythrocyte surface	$C1_s$	$C1_q$ and antibody	C4 and C2	$C4_a$ and $C'2_a$
Erythrocyte surface	$C2_a$	$C4_a$	C3	$C3_a$
TUA-particles	F_1	F_o	ATP (Rutamycin-sensitive)	$ADP + P_i$
Phospholipid – F_4	SDH	cyt. b	Ubiquinone Oxidised (TTB–sensitive)	Ubiquinone Reduced

paratopic interaction (from the greek para – next and topos – place). It is fundamentally different from allosteric interaction[2,3] in that it occurs at an interface only and from allotopic interaction[4] in that an interaction between proteins is essential.

Materials and methods. Paratopic interaction in general is recognized by a) solubilizing the intact system[16–19], which step can be omitted in the systems existing in blood plasma as they are already in a solubilized form. Then b) separation of the constituents[6–8, 11, 13, 16, 16, 20]. c) Reconstituting the system and estimating the activity as a function of the nature and amounts of components added[6, 7, 9, 10, 15–20]. For the experimental details, the reader is referred to the original literature.

Results and discussion. Perhaps the best documented of paratopic interactions is encountered in the generation of the enzyme prothrombinase, which catalyses the conversion of prothrombin (blood coagulation factor II) into thrombin. It has been suggested that prothrombinase consists of factor X_a and factor V_a (the subscript a denotes the activated factor) adsorbed next to each other onto a phospholipid surface. The evidence for this is in short: a) the minimal requirement for the generation of prothrombinase activity is the simultaneous presence of a phospholipid suspension, Ca ions and 2 proteins: the blood coagulation factor X_a and V_a[6]; b) when both proteins are bound to the same micelle, prothrombinase activity generates[7,8]; c) the kinetics for the formation of prothrombinase activity are in accordance with the model proposed[9].

In the prothrombinase complex, the active site is located in the factor X_a molecule, because a) pure factor X_a has a small but detectable prothrombinase action that can be increased 1000fold by addition of phospholipid and factor V_a. Phospholipid and factor V_a have no prothrombinase action either alone or in combination[10]; b) factor X_a is an esterase that can split synthetic esters (e.g. tosylargininemethylester) and that can be inhibited by diisopropylfluorophosphate. No enzymatic properties of factor V_a have been found[11].

Another example of paratopic interaction is the enzyme that converts factor X into its activated form via the intrinsic blood coagulation pathway. It consists of the coagulation factors IX_a and $VIII_a$ adsorbed onto a phospholipid micelle[12]. Factor IX_a is the active site here and factor $VIII_a$ is the paraenzyme. Paratopic interactions are not restricted to the blood coagulation reactions. The complement component $C1_s$ is a proesterase which, when bound to a cell surface via $C1_q$ and one IgM or 2 adjacent IgG antibody molecules, develops into an active esterase. The natural substrates of this esterase are the complement factors C4 and C2 that are converted into active forms. The latter 2 components are capable of combining and can also be adsorbed onto a cell surface. A surface bound enzyme then results that can convert still another complement factor viz. C3 into its activated form (see also the table[13–15]).

From the work of Racker and colleagues, it can be seen that paratopic interactions must also play a role in the composition of the enzyme system, catalyzing oxidative phosphorylation. In this work it is shown that among others the protein fraction F_1 and F_0 can be prepared from the inner membrane of beefheart mitochondria. F_1 has ATPase activity, but only when combined with F_0 and a particulate fraction from the inner mitochondrial membrane called TUA particles, this ATPase becomes sensitive to rutamycin (or oligomycin) as in the intact mitochondrion[16–18].

A 6th example can be found in the mitochondrion. Isolated succinate dehydrogenase (SDH) only accepts ubiquinone as a substrate and is only sensitive to inhibition by 4.4.4-trifluoro-1-(2-thienyl)-1.3-butanedione (TTB) when together with cytochrome b and particles consisting of phospholipids and coupling factor VI. The mode of interaction of active site carrier and para enzyme is unknown. Covalent bonding is unlikely to play a role, as most of the complexes described readily dissociate and recombine. Quaternary constraints analogous to these postulated by Monod et al.[3] in allosteric enzymes, but in this case asymmetrical, may be brought about in the active site carrier by the paraenzyme. The adsorption may serve as a means of orientating the molecules, and in itself may modify the tertiary structure of the protein moieties and hence influence their interaction.

Alternatively, for those allotopic complexes having a proteolytic action, one can imagine that little or no interaction between paraenzyme and active site carrier takes place, but that the substrate is more favourably bound to the complex than to the single enzyme. The observation by Esnouf that the esterolytic properties of factor X_a have not been enhanced by the formation of a prothrombinase complex hints in this direction[20].

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